Materials and methods. A diet of normal rat food (Purina Chow) containing 3000 ppm of elemental tellurium (K. and K. Labs., Plainview, N.Y.) was fed to 24 Wistar rats during gestation. These animals ate approximately 15 g of this diet per day. 20 rats gave birth to litters of hydrocephalic animals. 9 hydrocephalic new-born rats were anesthetized with ether and sacrificed. Brain tissues were fixed in 2.5% cold gluteraldehyde buffered with 0.1 *M* sodium cacodylate, pH 7.2, for 1 h, washed for 1 h in the buffer alone, postfixed with 1% osmic acid buffered with 0.1 *M* veronal acetate, pH 7.2, for 30 min, washed in the buffer alone for 1 h, dehydrated in graded alcohols and embedded in Epon 812. Tissues from 3 of these brains were not postfixed in osmic acid. Thin sections were cut and subsequently stained with 1% uranyl acetate followed by 1% lead citrate (Reynolds), carbon-coated and examined with an RCA EMU4 electron-microscope. The tissues for examination by light microscopy were stained with hematoxylin-eosin or cresyl violet (Nissl).

Observations. Paraffin and celloidin sections of the cerebral tissues examined with the light microscope did not reveal intracellular deposits. Examination with the electron-microscope of cells in postfixed and nonpostfixed tissues in the cerebral cortex and ependyma showed small dark particles within lysosomes in the cytoplasm of some neurons and glial cells. Such particles were not free in the cytoplasm. As these electron-dense particles were present in stained and unstained sections of postfixed and nonpostfixed tissues, it was presumed that they were neither lipofucsin nor glycogen.

Discussion. The purpose of this communication is to report the presence of anomalous electron-dense particles in the lysosomes of neurons and glial cells in the brains of new-born rats with tellurium-induced hydrocephalus. Similar electron-dense bodies have been identified in the neurons and glial cells of adult animals intoxicated with tellurium[7]. It is therefore suggested that the dark bodies reported here are also telluric in nature.

The importance of identifying the presence of tellurium in the hydrocephalic brain is that this would demonstrate the presence of the teratogenic agent directly in the tissues. GARRO and PENTSCHEW[10] noted similarities of the hydrocephalus caused by tellurium and that caused by vitamin B_{12} deficiency and referred to vitamin-imbalance and metabolic-disorder in attempting to explain the cause of tellurium-induced hydrocephalus. The work done with autoradiography[9,12] and the present observations show that the tellurium is present in the cerebral tissues.

The reason for the macroscopic appearance of the tellurium-induced non-hydrocephalic 'black brain' of adult animals was the large doses of tellurium given[2-4]. The absence of macroscopically visible dark deposits in the hydrocephalic brains of the developing rats is presumably because of the very small doses of tellurium given.

Résumé. L'étude présente concerne la localisation du tellure dans le cytoplasme de cellules nerveuses fœtales et post-natales chez des animaux dont la mère a ingéré du tellure durant la grossesse.

S. DUCKETT and T. SCOTT

Department of Neurology, Jefferson Medical College, Philadelphia (Pennsylvania 19107, USA),
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Oncogenicity of a Cell Line Derived from Adenocarcinoma of the Salivary Gland of C3H/He Mouse

The data concerning the occurrence of viral particles in cultured neoplastic cells and their modifications are somewhat discrepant. Especially as far as Bittner virus is concerned, it has been found that the cells isolated from the mammary tumor show a different morphological pattern in relation to the presence of viral particles[1,2].

In the course of a systemic study on the properties of mouse salivary gland adenocarcinoma[3], induced by a Bittner-like virus[4], investigations have been made on the behaviour of cultured cell lines correlated to the modifications occurring during the maturation of the viral particles.

The tumor was removed on 18th day, freed from the necrotic areas, minced and trypsinized at +4 °C overnight. The cells were washed 3 times with the growth medium and suspended at concentration of 100,000 cell/ml in Eagle basal medium supplemented with addition of 10% of inactivated calf serum. The pH was adjusted to 7.2 by CO_2 flowing. Subcultures were prepared by the standard method and the morphological features were studied by conventional methods. Occasionally the cell function was checked by measurements of O_2 consumption.

The cultured cells grew rapidly and gave an uniform and complete monolayer. In the primary culture the cells sheet was obtained within 72 h, while for the subsequent passages the time was longer (5–6 days). At the 4th subculture symptoms of growth modifications were observed. Essentially the cultures lost their monolayered aspect and the cells clumped as isolated colonies, being epithelial, nevertheless. When analyzed at light microscopy, they maintained their morphology.

The non-subcultured cells suffered regular cycles of an almost complete destruction and a partial reconstitution of the cellular layer originating from the surviving cells. After this time the cultures appeared to be completely destroyed; nevertheless the bottles were kept at 37 °C and the growth medium changed weekly. 97 days after the first passage, a clonal type growth was observed and on the 120th day the cells were completely monolayered. These cells were tested for their oncogenicity in C3H/He Ar/IRE mice by injecting s.c. different quantities (Table).

[1] Y. TSUBURA, K. TOKYOSHIMA, S. SANO and T. WATANABE, *Cancer Cells in Culture* (1968), p. 216.
[2] J. A. SYKES, J. WITHESCANVER and L. BRUGGS, J. natn. Cancer Inst., USA *41*, 1315 (1968).
[3] A. CAPUTO and L. ORCI, Z. Krebsforsch. *73*, 46 (1969).
[4] L. CASTELLI and A. CAPUTO, Experientia *26*, 780 (1970).

As reported in the Table, the cells were oncogenic in isogeneic hosts until 180th day, and the concomitant occurrence of intra and extra cellular Bittner-like particles was ascertained. After the 233th day the cells were no more able to induce tumors in C3H/He Ar/IRE although their inoculum size was 20 times larger. By electron microscopy it has always been possible to correlate the lost of oncogenicity to the absence of viral particles.

Relationship between the occurrence of virus particles and the oncogenicity observed for salivary adenocarcinoma cell cultured in vitro

Age of the culture	No. of cells inoculated	Site of inoculation	Tumor takes in C3H/He strain	Intracellular particles/cells[a]
20 days	40,000	s.c.	100% (30/30)	116
45 days	40,000	s.c.	100% (30/30)	128
100 days	40,000	s.c.	80% (24/30)	102
121 days	80,000	s.c.	70% (21/30)	73
180 days	80,000	s.c.	70% (21/30)	60
233 days	800,000	s.c.	0% (0/30)	0
253 days	800,000	s.c.	0% (0/30)	0
253 days	800,000	i.p.	0% (0/30)	0

[a] Each value represents an averaged figure obtained counting 10 different cells.

On the basis of these results we can conclude that the virus is the causative agent of the salivary gland adenocarcinoma. Experiments are now in progress on the relations of the virus and on its characteristics, in order to know its most important properties and any possible differential character toward typical Bittner-virus.

So far as the observations concerned the mammary tumor, it has been reported[1,2,5] that the virus-free cells when injected in virus-carrier animals are able to assume again the host's virus particles. In contrast to this from the data above reported, it seems that the cells isolated from the salivary gland tumor are unable to utilize viral particles occurring in the host in order to replace their original oncogenicity.

Riassunto. Cellule portatrici di virus derivate da adenocarcinoma della ghiandola salivare coltivate in vitro sono oncogeniche per l'ospite isogenico per circa sei mesi di coltura. Dopo tale periodo perdono l'oncogenicità e non è più possibile ritrovare particelle virali. Sulla base di questi risultati si conclude che il virus è l'agente causale dell'adenocarcinoma della ghiandola salivare del topo C3H/He.

MARIA L. MARCANTE, L. CASTELLI and A. CAPUTO

Regina Elena Institute for Cancer Research, Viale Regina Elena 291, Roma (Italy), 21 October 1970.

[5] K. K. SANFORD, *Cancer Cell in Culture* (Ed. H. KATSUTA; University of Tokyo Press, Tokyo 1968), p. 281.

Growth Promotion by Extracts from Wilms' Tumor in vitro

We have recently isolated a relatively homogeneous protein-polysaccharide complex from Wilms' tumor which resembles an abnormal serum component detected in Wilms' tumor-bearing children[1]. The tumor component was isolated by a procedure previously used to obtain acid mucopolysaccharides (AMPS) from the cell surface of normal and polyoma-virus transformed fibroblasts in culture[2]. The fibroblast AMPS extracts promote or inhibit cell proliferation depending on whether they are derived from viral-transformed or normal cells, respectively. We explored, therefore, the possibility of a similar activity in AMPS cell surface materials derived from Wilms' tumor, and in serum from Wilms' patients.

The abnormal component was isolated from sera of Wilms' patients by adding 1 volume of 6% acetic acid per volume of serum and separating the supernate by centrifugation at $5000 \times g$. The precipitate was washed in 3% acetic acid and redissolved in 3 volumes of pH 8.5 buffered isotonic saline. Both serum precipitate (SP) and serum supernate (SS) were assayed for growth effects on cells in culture.

A portion of fresh Wilms' tumor was washed with saline, minced, and extracted with 0.02% disodium EDTA in calcium- and magnesium-free phosphate buffered saline. About 400 g of minced tumor was stirred with 300 ml EDTA solution in a spinner culture flask. After 1 h, this suspension was centrifuged ($5000 \times g$, 15 min), the viscous supernate was diluted 1:2 in saline, and made up to 3% acetic acid by volume, as with the serum samples. The resulting tumor precipitate (TP) and its supernate were also tested in growth studies.

A 0.1 ml aliquot of each test solution was added to a series of Leighton tubes containing known concentrations of trypsinized test cells in 1.5 ml of Eagle's minimum essential medium (MEM) supplemented with 10% newborn calf serum, and penicillin and streptomycin (Grand Island Biologicals). Control tubes were prepared from the same pools of cells, but contained 0.1 ml of media or normal human serum in lieu of serum or tumor

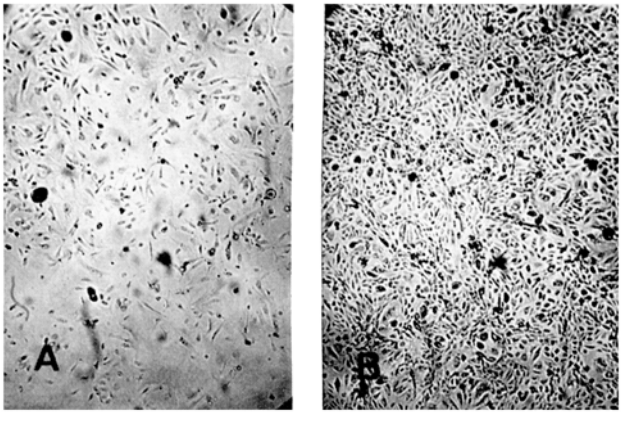

Fig. 1. Primary human embryonic kidney cell cultures in Leighton tubes after 105 h in culture (× 50). A) Control cells; with 0.1 ml normal human serum added. B) Test cells with 0.1 ml of an EDTA extracted Wilms' tumor fraction added (AMPS content; 27 µg determined as hexosamines plus uronic acids).